Special Lecture

Sex Determination and Gonadal Sex Differentiation in Fish

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Sex determination and gonadal sex differentiation in fish are plastic. Since its first discovery in the medaka, *Oryzias latipes*, sex reversal using exogenous steroid hormones around the time of sex determination has been duplicated in other species. Because of these features, the fish is an excellent model for the study of endocrine disrupting chemicals. We have used three major fish species to study the genetic, hormonal and environmental aspects of sex determination and gonadal sex differentiation in fish.

Nile tilapia, *Oreochromis nitolicus*, is an excellent example of the precise nature of steroidogenic actions during gonadal sex differentiation. Using all genetic male and female tilapia, we have shown that steroid-producing cells in ovaries prior to and during sex differentiation express all of the steroidogenic enzymes required for estradiol-17β production. These results, together with evidence of masculinization of genetic females by fadrozole or tamoxifen, strongly suggest that endogenous estrogens act as the natural inducers of ovarian differentiation in tilapia. In contrast, the ability of steroid-producing cells to synthesize steroid hormones in all-male fry appears after testicular differentiation. *DMRT1* is expressed male-specifically in testicular Sertoli cells during sex differentiation, suggesting an important role of *DMRT1* in testicular differentiation in tilapia.

Protogynous hermaphroditism (i.e. female to male sex change) exemplifies the plasticity of teleost sex determination and sexual differentiation. Sex change takes place following alteration of social dominance, such being dependent upon differences in relative body size. In *Thalassoma duperrey*, down-regulation of gonadal aromatase correlates with ovarian degeneration during sex change indicating estrogens maintain female function and development. The subsequent sex change, i.e. testis formation, may be the default pathway in the absence of estrogens, the result of up-regulation of or increased responsiveness to putative testis-determining factors (e.g. androgens, DMRT1), or some combination.

The medaka has two major advantages for genetic research: a large genetic

diversity within the species and the existence of several inbred strains. Using positional cloning and detailed sequence analysis of BAC clones by shotgun sequencing, we have recently identified *DMY* (DM-related gene on the Y chromosome) as a strong candidate for the sex-determining gene of medaka, which possess a stable genetic XX/XY sex determining system. *DMY* contains the highly conserved DM domain found in other genes involved in sexual development in both vertebrates and invertebrates. Expression analysis during early embryogenesis shows that *DMY* is found in the somatic cells (Sertoli cells) of the XY gonads at the time when sex determination occurs. Two naturally occurring XY female mutants established *DMY*'s critical role in male development. One of these mutants contained an insertion that causes premature termination of the DMY protein. When mated, all of XY offspring with the mutant Y were female. The other mutant had a severe depression in *DMY* in the embryo and 60% of its XY offspring with the mutant Y developed as females. *DMY* provides the first example of a sex-determining gene in non-mammalian vertebrates.